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ROCHESTER INSTITUTE OF TECHNOLOGY

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Fine and Applied Arts in Candidacy for the Degree of
MASTER OF FINE ARTS

Rheumatoid Arthritis and Myasthenia Gravis as
Examples of Autoimmune Diseases

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Introduction

Autoimmunity is an inappropriate immune response against an individual's own healthy body tissues. A wide variety of autoimmune diseases have been identified, and they affect different tissues in different ways. Some are very specific to particular organs, while others are more broad ranging in scope.

One example of an autoimmune disorder is myasthenia gravis. This disease affects the neuromuscular junction. The receptors of the skeletal muscle cells are blocked and degraded by antibodies. The result is a gradual weakening of the patient's limbs, as nervous stimulation of the muscle is inhibited.

Another example of an autoimmune disease is rheumatoid arthritis. The predominant feature of this condition is inflammation of the synovial joints throughout the body. Accompanying the inflammation is the destruction of articular cartilage and the bone beneath it.

This thesis will focus upon rheumatoid arthritis and myasthenia gravis as examples of autoimmune diseases. It consists of twelve full color illustrations. Their intended use would be as reproductions in an undergraduate textbook on immunology. I have chosen to concentrate upon the cellular and chemical interactions of these pathologies.

In order to properly understand the pathophysiology of an autoimmune condition, a full discussion of the normal immune response is necessary to provide background. This discussion begins with a definition of what provokes an immune response. The immune system is charged with the task of monitoring all internal regions of the body for foreign substances. When a foreign substance is detected, it is attacked by an immune response.

The normal immune system distinguishes a healthy structure that is supposed to be in the body from a foreign and potentially threatening substance; in other words, it can distinguish “self” from “non-self.” Any molecule which is capable of inducing an immune response is termed an antigen (The New Penguin Dictionary of Biology, 1990).

In order for a molecule to provoke an immune response, a portion of it must consist of a series of chemical elements which act as a code. The code is “read” by the immune system and interpreted as foreign. This precise portion of the antigenic molecule is called either the epitope or antigenic determinant (Barrett 1988, 38; Tizard 1988, 18) and is the part of the antigen which will interact directly with the immune system.

The antigenic determinants are structured in accordance with genetic instructions. The group of genes which defines the distinctive antigenic determinants of self antigens is called the major histocompatibility complex.

When an immune response is mounted against an invading organism, such as a bacterium, it is not mounted against the entire organism. Rather, it is mounted against various antigenic determinants on the molecules which compose the bacterium. There may be a variety of different antigens upon a single bacterium, each provoking an immune response (Tizard 1988, 30).

Viruses and pollen grains are two other common sources of antigens. However, to be identified as a foreign antigen it is not necessary for the insulting substance to originate from outside the body. Cancer cells originate from within the body and, if significantly different from normal cells, will be interpreted as foreign and attacked (Fig. 1).

The germinal event in an immune response is phagocytosis of the foreign body by cells of the mononuclear phagocytic system (MPS). The

mononuclear phagocytic system is a component part of the immune system, and is composed entirely of mature macrophages (Roitt 1991, 4).

Macrophages are found throughout the body, but are particularly prevalent in the lung, liver, lining of spleen sinusoids and lymph node. Macrophages may be known by another name when found in particular tissues. For example, a macrophage within the liver is termed a Kupffer Cell (Roitt 1991, 4). Macrophages originate from promonocytes within the bone marrow and then drift through the circulatory system as monocytes. When they are mature they migrate into tissues as macrophages (Barrett 1988, 86).

A macrophage may adhere itself to a free surface, such as the lining of a blood vessel, and wait for an antigen. Alternatively, it may be chemically attracted to an antigen from some distance away, in which case it will move against the antigen.

Once an antigen is detected, the macrophage will endeavor to bind the antigen to keep it from getting away (Barrett 1988, 100). Opsonins are chemicals in the body which coat antigens to make attachment by macrophages or other phagocytic cells easier. Some examples of opsonins are antibodies, fibronectin, and the complement protein C3b. Opsonins bind to antigens and then lock into receptors upon macrophages. This linkage makes attachment of the macrophage to the antigen much more efficient.

There is a tendency for particles or cells suspended in body fluids to take on a negative charge (Tizard 1988, 43). If both the antigen and phagocytic cell have negative charges, they will repel each other slightly. Opsonins aid in attachment by neutralizing any negative charge on the antigen (Tizard 1988, 45).

After the attachment is accomplished, the macrophage surrounds the antigenic substance and seals it within a vacuole. The antigen is moved towards the macrophage's center while lysosomes provide enzymes to kill it (Barrett 1988, 101).

Another part of the immune system is what is called humoral immunity. Humoral immunity involves antibodies circulating in body fluids (Webster's Medical Desk Dictionary, 1986). An antibody is an immunoglobulin, or a globulin molecule, produced in response to an antigen (Barrett 1988, 25). There are five distinct classes of immunoglobulins. The most well-known and best studied is the IgG class (Barrett 1988, 48). IgG has a molecular weight of 150,000 daltons, and consists of two pairs of polypeptide chains. One pair, the heavy pair, is arranged in parallel and bound together by disulfide bonds. Towards one end, the heavy chains diverge, forming a "Y" shape. Adjacent to both divergent upper segments of the "Y" shape is a light chain, one on each side. It is the top two ends of the "Y" shape which bind antigen (Keeton 1986, 775). Antibodies play a central role in the immune response, so it may not be surprising that antibodies play a central role in many autoimmune diseases.

Plasma cells are the producers of antibodies. Plasma cells are derived from B-lymphocytes, as part of the immune response (Fig. 2). B-lymphocytes are a type of white blood cell. Each B-cell is specifically designed to react with one specific antigen. No other antigen will have any effect upon a B-cell except that one particular antigen for which it is specific. Contact with an antigen is the first step in bringing about the maturation of the B-cell into a plasma cell (Tizard 1988, 202). B-cells have immunoglobulin molecules scattered across their surface to act as antigen receptors (Barrett 1988, 116).

B-cells will sometimes require more than just contact with antigen if they are to differentiate. A cell which mediates the immune response, known as the helper T-cell, is sometimes necessary. Helper T-cells must have antigen presented to it by a cell, such as a macrophage, with MHC class II molecules in order for it to activate (Sheehan 1990, 31). Once activated, the helper T-cell produces lymphokines. These lymphokines include B-cell growth factor (BCGF) and B-cell differentiation factor (BCDF), both of which assist in promoting the maturation of B-cells to plasma cells (Tizard 1988, 204). Helper T-cells also secrete a variety of lymphokines which increase the effectiveness of macrophages (Tizard 1988, 244).

There are some antigens which will provoke B-cell activation and differentiation without the help of a helper T-cell. These antigens are classified as T-cell independent antigens (Sheehan 1990, 34).

Myasthenia Gravis

Myasthenia gravis is an autoimmune disease which is chiefly characterized by increased fatigability of skeletal muscle. Typical symptoms include slurred speech, nasal voice, drooping eyelids and weakness of the proximal extremities. The condition is most commonly seen in young adults (Bullock 1992, 1092). The specific focus of the disease is the acetylcholine receptors of the neuromuscular junction.

Each individual muscle cell of the body requires direct nervous innervation. This innervation comes from a branch of a nerve fiber which does not physically contact the muscle cell. Rather, the terminal end of the nerve lies within an indentation in the muscle cell, called the neuromuscular

synaptic cleft (Fig. 3). The terminal end of the neuron is almost completely surrounded by the muscle cell. The terminal end of the neuron and the small portion of the muscle cell which encircles it is called the motor end plate. The neuron is separated from actually touching muscle by a small gap called the synaptic cleft (Ross, Reith and Romrell 1989, 208). The nerve may stimulate the muscle to contract by releasing acetylcholine, which diffuses across the synapse to bind with receptors on the muscle.

The surface of the muscle cell which faces the neuron across the synapse is not a smooth concave surface. Rather, the surface is broken by creases arranged parallel to each other. These crevices are termed subneural clefts. The regions of this undulating surface which form high plateaus between the subneural clefts are called the junctional folds (Ross, Reith and Romrell 1989, 210). Located upon these junctional folds are the acetylcholine receptors (Seybold 1991, 77). The acetylcholine receptors are protein molecules that are embedded in the bilipid membrane of the muscle cell. The receptors are 7.8 Angstrom units at their widest points. Their total height is 11.5 Angstrom units, with only 4 Angstrom units of it appearing on the exterior of the muscle cell (Salpeter 1987, 296).

These receptors change shape when acetylcholine binds to them, allowing a channel to open between the synaptic cleft and the interior of the muscle cell. This causes a change in the electrical charge of the muscle cell as sodium ions stream into the cell. If strong enough, a shift in polarity will be propagated along the muscle fiber and stimulate it to contract (Seybold 1991, 71). When the neuron releases acetylcholine, it usually releases more acetylcholine than is necessary to depolarize a muscle. However, acetylcholine is normally broken down very quickly into its two component

parts: acetic acid and choline. This breakdown is implemented by the enzyme cholinesterase, a substance normally present in the synapse (Seybold 1991, 71). After this transpires, the acetic acid and choline move back to the neuron to be reabsorbed and reconstituted (Fig. 4).

In the condition known as myasthenia gravis, antibodies produced by the immune system block and degrade acetylcholine receptors. Exactly what causes this unfortunate event is at this time unclear. It has been hypothesized that some unknown antigen may enter the body possessing antigenic determinants which are very similar to acetylcholine receptors (Vincent 1990, 182). Thus, the antibodies generated in the ensuing immune response would cross react with the acetylcholine receptors (Fig. 5). Of all patients with generalized Myasthenia gravis, 90% test positive for circulating antibodies specific for acetylcholine receptors (Seybold 1991, 72).

How the antibody blocks the binding of acetylcholine to the receptors remains undetermined. The receptors are composed of subunits arranged like staves of a barrel (Seybold 1991, 71). These have been designated alpha, beta, gamma and delta. The alpha component is repeated twice, making for a total of 5 subunits (Salpeter 1987, 296). Acetylcholine binds to sites located on the two alpha components. Blockage of this binding site may not be due to the antibody binding to the identical spot where acetylcholine is supposed to bind. In the majority of cases antibodies bind to other locations on the receptor, but overlap and block the acetylcholine binding site. This is called steric hindrance (Drachman et al. 1987, 93). Alternatively, the antibody may bind directly to the location on the receptor which binds acetylcholine. Or, it may bind to the receptor in a way which could prevent the opening of the central ionic channel (Seybold 1991, 72).

Acetylcholine receptors are constantly recycled by the muscle cell. Every 10 to 14 days they are endocytosed and replaced. Antibodies bound to acetylcholine receptors are capable of fixing blood complement proteins (Tizard 1988, 523). The formation of complement into membrane attack complexes may disrupt the muscle cell's membrane and interfere with receptor replacement. The degree to which this process contributes to the disease remains controversial (Seybold 1991, 72).

Anti-acetylcholine receptor antibodies may also degrade the number of receptors by accelerating their endocytosis. This process relies on the ability of antibody molecules to bind to more than one antigen at a time. Receptors may become cross linked by antibodies and drawn together into tiny clusters on the membrane surface, where they are endocytosed in groups and enzymatically degraded with enzymes from lysosomes (Fig. 6) (Drachman et al. 1987, 92; Dixon and Fisher 1983, 277).

One can now comprehend how the transmission of nervous impulses is impeded from stimulating the muscle fibers during myasthenia gravis. Acetylcholine is released normally into the synapse. However, the total numbers of receptors available are reduced. This is due to receptor blockage from antibodies as well as complement-mediated damage and accelerated endocytosis of the receptors. Therefore, not enough ions are released for depolarization of the muscle to occur, and the muscle will not contract. The result is abnormal muscle weakness and fatigability (Bullock 1992, 1092).

There is no cure for myasthenia gravis, but effective treatments are available. This usually begins with dosages of anticholinesterase medications, such as pyridostigmine bromide, ambenonium chloride or

neostigmine bromide (Seybold 1991, 84). These medications will inhibit the enzyme cholinesterase from breaking down acetylcholine into acetic acid and choline. With extra intact acetylcholine in the synapse, it is more likely that the few receptors remaining will bind to the neurotransmitter. Hence, depolarization will be sufficient to cause muscle contraction. However, over dosage of anticholinesterase may cause increased fatigability due to desensitization of the postsynaptic membrane (Seybold 1991, 96). Other therapies include corticosteroids, plasmapheresis and azathioprine (Seybold 1991, 86). In 12% of all cases, hyperplasia of the thymus is evident, sometimes requiring thymectomy. The prognosis for patients with myasthenia gravis utilizing the current therapies available is generally good, with the caveat that they may be required to avoid extremely fatiguing activities (Seybold 1991, 84).

Rheumatoid Arthritis

The most common clinical manifestations of rheumatoid arthritis are pain and swelling of joints in the extremities. The small joints of the hands and feet are the most likely to be affected, but involvement of the knees, ankles, elbows, wrists and hips are not infrequent. Some patients experience occasional flare-ups and remissions, while others endure a constant attack of fluctuating intensity (Samter et al. 1988, 1365). Rheumatoid arthritis is three times more common in women than men. There is a direct correlation between aging and the frequency of the disease (Samter et al. 1988, 1366). Studies of twins and familial groups show that genetic factors play a role in predisposing individuals to developing rheumatoid arthritis (Samter et al. 1988, 1367).

The synovial joints are a typically involved region of the body in rheumatoid arthritis. Therefore, it is necessary to briefly define the anatomy of a healthy synovial joint (Fig. 7). First, the articulating surfaces of the bones are covered with hyaline cartilage. Hyaline cartilage is composed of a homogeneous matrix surrounding small dispersed spaces called lacunae. Within these lacunae live chondrocytes, which are specialized cells responsible for the production of matrix. The matrix is composed of two materials, collagen fibrils and ground substance (Ross, Reith and Romrell 1989, 124). The collagen fibrils are formed like archways with the highest point of the archway adjacent to the free articulating surface and the two anchor points of the archway down adjacent to the bone (Weiss 1983, 251). The collagen fiber arrangement can also be compared to a three dimensional felt-like pattern. The ground substance of hyaline cartilage is composed of proteoglycans, glycosaminoglycans and glycoproteins (Ross, Reith and Romrell 1989 123).

Surrounding the joint is a capsule of connective tissue called the joint capsule. The area enclosed by the capsule is called the joint space and it is filled with fluid. The joint capsule is vascularized tissue, composed of both dense and loose connective tissue. The interior lining of the joint capsule is the synovial membrane, composed of a one to three layer thick surface of cells termed synoviocytes. These synoviocytes are categorized into three types. The first type is simply a precursor for the other two, and is called a type C cell. The second sort of synoviocyte exhibits macrophage-like phagocytic behavior and is known as the type A cell. The last cell is called type B. It is responsible for the secretion of the fluid which fills the joint space (Firestein, Tsai and Zvaifler 1987, 449).

There are two characteristics which make it easy for substances to pass into the joint space. First, the capillaries within the joint capsule are highly fenestrated. Also, the layers of synoviocytes possess no true basement membrane between them and the adjacent connective tissue (Utsinger, Zvaifler and Ehrich 1985, 153).

The antigen which initiates the long chain of events known as rheumatoid arthritis has not yet been identified. It is believed that this unidentified antigen enters the body and provokes an immune reaction. However, the antibodies produced in the immune reaction are somehow altered. Possibly this damage may be due to an enzyme in the inflammatory process. This alteration of the antibody molecule reveals a new antigenic determinant. Some genetically predisposed individuals will mount an immune response against this newly created antigen, generating antibodies against antibodies, or rheumatoid factor (Fig. 8) (Utsinger, Zvaifler and Ehrich 1985, 152).

Hence, these antigenic antibodies will become chemically bound to rheumatoid factor and form immune complexes. These immune complexes tend to settle within soft connective tissues and hyaline cartilage (Utsinger, Zvaifler and Ehrich 1985, 151). As immune complexes aggregate, they activate the complement cascade in what is called a type III hypersensitivity (Tizard 1988, 490).

Current dogma breaks rheumatoid arthritis down into two phases: the exudative phase and chronic phase. The exudative phase is focused on polymorphonuclear cells in the joint space and changes in the cells lining the joint capsule. The chronic phase centers upon interactions between immune cells within the joint capsule (Ziff 1990, 127).

Blood complement will increase vascular permeability allowing serum proteins and cellular blood elements into the region. The first observable histological change in a synovial joint is perivascular infiltration of inflammatory cells around the small blood vessels of the capsule, along with vessel dilation (Samter 1988, 1369). Blood complement will act as a chemotactic agent for neutrophils, which then move into the synovial joints to phagocytose the antigen complexes (Fig. 9).

Under ideal conditions, neutrophils first engulf the immune complex before releasing digestive enzymes (Tizard 1988, 44). Unfortunately, the location of aggregated immune complexes in rheumatoid arthritis is far from ideal. The complexes often settle within hyaline cartilage. This is understandable, considering that between 60 to 78 percent of hyaline cartilage's net weight is water (Ross, Reith and Romrell 1989, 123). Though most of this water is bound, some is bound so loosely as to allow materials, such as immune complexes, to settle within the cartilage matrix. Therefore, neutrophils are unable to internalize the sequestered immune complexes, but may still be able to make contact and bind with them. Under these circumstances, neutrophils release the contents of their lysosomes directly onto the cartilage. These contents include neutral proteinases and collagenase which may damage the cartilage by degrading collagen fibrils and proteoglycans (Fig. 10) (Roitt 1991, 333).

Another complication involving neutrophils is the release of cellular contents which occurs upon cell death. These contents include kininogens and vasoactive amines which promote vascular dilation and edema. Other enzymes promote mast cell degranulation of histamine when released, thus causing more edema (Utsinger, Zvaifler and Ehrich 1985, 154). Also, type A

cells of the synovial lining phagocytose immune complexes, releasing inflammatory mediators in the process. They release enzymes such as acidic and neutral proteinase which attack proteoglycan (Utsinger, Zvaifler and Ehrich 1985, 100).

Another observed phenomenon is the enlargement of the lacunae within the cartilage. It has been theorized that chondrocytes may produce enzymes which degrade the cartilage matrix. Chondrocytes also tend to proliferate during rheumatoid arthritis (Utsinger, Zvaifler and Ehrich 1985, 155).

One hallmark of chronic rheumatoid arthritis is pannus formation. Pannus is the ingrowth of synovial tissue into the joint. It is unclear precisely what is the stimulus for this, but lymphokines from helper T-cells seem the most likely candidate. There are three known types of pannus. The first type appears as immature synovial cells multiplying and advancing across cartilage from the recesses of the marginal edge of the cartilage. They also insert themselves between the collagen fibers and proliferate. Thus, the cartilage is attacked from both outside and from within. Pannus showing these characteristics is classified as active pannus. There is also a highly cellular form of pannus which appears as mature granulation tissue, including fibroblasts, inflammatory cells and blood vessels. A third type is dense, avascular and acellular, and it is capable of killing cartilage by blocking its nutrition. It is unclear whether or not these three types of pannus occur sequentially or independently (Fig. 11) (Utsinger, Zvaifler and Ehrich 1985, 155).

During the chronic phase, blood complement may act as a chemotactic factor for macrophages, which then move into the synovial joints to

phagocytose antigen complexes. Macrophages then present the pathogenic material to lymphocytes, which will in turn interact to produce more antibodies which add to the aggregation of immune complexes (Utsinger, Zvaifler and Ehrich 1985, 153).

Besides antigen presentation, macrophages influence lymphocytes through the release of interleukin-1. Interleukin-1 causes T-cells to release interleukin-2 and B-cell growth factor, which stimulate the growth of B-cells. Interleukin-1 also promotes the release of destructive enzymes by dendritic cells of the synovium (Fig. 12) (Utsinger, Zvaifler and Ehrich 1985, 156).

Not only do macrophages act in an excitatory way on helper T-cells, but helper T-cells promote macrophage activity through the release of lymphokines, such as macrophage aggregation factor and macrophage stimulating factor (Utsinger, Zvaifler and Ehrich 1985, 156).

Some patients with rheumatoid arthritis have been found to have antibodies to collagen circulating in their blood. This may indicate that an immune response mounted against damaged cartilage may play a role in perpetuating the immune response (Samter et al. 1988, 1389). However, it is believed the primary factor in perpetuation of rheumatoid arthritis is that macrophages stimulate lymphocytes which then stimulate macrophages and so the chronic inflammation continues in an endless positive mutual feedback system (Utsinger, Zvaifler and Ehrich 1985, 157).

It is possible that the cellular interactions involved with the perpetuation of rheumatoid arthritis may be involved during the routine turnover of connective tissue during differentiation, growth and repair. But possibly some vital control mechanism for this natural process may be defective, causing the perpetuation of the pathology. (Utsinger, Zvaifler and Ehrich

1985, 157)

Rheumatoid arthritis can not be cured, but treatment plans may be implemented to reduce pain and swelling while maintaining mobility. Rest is recommended to reduce stress on afflicted joints. Exercise is useful for maintaining joint mobility and muscle tone (Porth 1990, 1107). Inflammation may be reduced by the administration of aspirin and other nonsteroidal anti-inflammatory drugs (Porth 1990, 1108). Rheumatoid arthritis seems to be modifiable with immunosuppressant drugs, though as of yet only azathioprine is FDA approved. If inflamed synovial tissue is unresponsive to drug therapies, then surgical removal of the synovium, or synovectomy, may be performed. Other surgical treatments include repair of damaged tendons. In extreme, cases total joint replacements with prosthetics will increase mobility and reduce pain (Porth 1990, 1109).

Conclusion

Myasthenia gravis and rheumatoid arthritis are both examples of autoimmune diseases, a category of ailments involving a mistaken attack on healthy tissues by the immune system. Myasthenia gravis causes harm through cross reactivity between an antigen and acetylcholine receptors. Rheumatoid arthritis is in a category of autoimmune diseases known as immune complex diseases, which cause lesions through the activity of leukocytes and blood complement. Neither of the antigens which trigger these conditions has been identified and no cures have been found.

Technique

At all times I tried to illustrate the various cells as they might look while alive and inside the body, rather than as they appear in slide preparations. Scanning electron micrographs proved a valuable source, though some cells may appear distorted due to artifacts left by processing the specimens.

My choices of color were based on aesthetic judgment, tempered by consideration of what color a student might be used to seeing the tissue in his textbooks. For example, I chose light blue as the color for hyaline cartilage because that is what color it appears in Frank Netter's medical illustrations. I chose shades of pink to represent the loose and dense connective tissue of the joint capsule because that is how it appears when stained with hematoxylin and eosin.

My technique was fairly consistent throughout the 12 illustrations. I would first sit down with a pad of drawing paper and a pencil and make a few rough sketches. Once I had something that I was reasonably happy with, I would go into the computer lab and begin drawing the outlines of the objects with Adobe Illustrator. I chose not to utilize the Adobe Streamline application to trace my sketches because they were drawn roughly. Drawing with the pen tool in Adobe Illustrator allowed me to further refine my drawings and experiment with composition.

Once the outline was completed I brought it into Photoshop without anti-alias. The reason for this was that outlines without anti-alias were much easier to select with the magic wand tool since they were composed of adjacent pixels of identical color. The next step was filling in the areas delineated by the outlines with blends or fills. If a blend and a fill had to be changed it was much easier to select if the outlines were left black until all

were complete.

In the illustration entitled What is an antigen?, the forms were simple so outlining in Adobe Illustrator seemed unnecessary (Fig. 1). For the pollen I created a tan circle, and then added the shadows and highlights with the airbrush tool. The crevices in the pollen granules were added with the pencil tool then smoothed and blended with the finger tool. The viruses were drawn with a frame work of straight lines, then the highlights were added with the airbrush.

The cancer cells were B-lymphocytes copied out of the illustration I had drawn of the immune response. I changed the color of the cell from magenta to green using the color control menu, then added more texture. The bacteria were also extracted from the immune response illustration.

The background surface of the illustration depicting the immune response was created in two steps (Fig. 2). I first drew a small portion of the texture initially as a separate document. I then utilized the rubber stamp to clone the texture into the background. I did not want the background to appear as a wall of identically textured tiles, so I would frequently stop the cloning process, reselect a different portion of the original texture and start cloning again.

A large portion of the yellow macrophage's texture was drawn with the drawing tools, then cloned using the same start and stop method used for the background. I added the core shadow with the airbrush tool.

The lymphocytes were constructed by making a radial blur in a selected circular area, then adding reflected light with the airbrush tool and a dab of white with the paintbrush tool adjusted to 50% opacity to intensify the highlight. The texture was drawn with the pencil tool. The plasma cell had

to be drawn with the paint brush and airbrush tool since the radial blend only works in a perfect circle. The nucleus was a radial blend drawn on a separate document and pasted into the cell at 50% opacity. The rough endoplasmic reticulum was drawn by hand with the pencil tool. All antibodies throughout the thesis are the same antibody duplicated, resized and rotated. The bacteria were filled with solid red color, then shadows, highlights and details were added with the drawing tools.

The anatomy illustration of the synovial joint was relatively straightforward (Fig. 7). Everything was drawn as a flat color or blend, except for the texture of the bone which was drawn as a small portion, then duplicated to fill the space.

The neutrophils appearing in the illustrations were not drawn with fills or imported outlines (Figs. 9 and 10). Instead, I delineated the edges of the form with the pen tool and converted it to a selection. Then with the airbrush tool, I selectively added purple shadows and white highlights while leaving a portion of the background behind the cell still visible. The nuclei and the arterioles were rendered with the various drawing and painting tools.

In the illustration representing a neutrophil eating through collagen, the fibers were drawn with lines of various shades of blue, each one pixel wide (Fig. 10). Some fibers were then rotated to appear broken.

The panoramic landscape view of the cartilage undergoing destruction began as a relatively refined pencil drawing, which was then scanned on a flat bed scanner and opened in photoshop (Fig. 11). First the image was recomposed by moving and distorting various areas. This was purely for aesthetic reasons. Then a blend from blue to red was added to the background from left to right. Then the surface plane was filled with a blend,

allowing it to lighten as it neared the horizon line. I wanted to maintain the pencil texture in the canyon and pannus. So, I selected the largest diameter for the paintbrush tool but set it to color only, and then proceeded to “colorize” those areas.

The explosion from the bursting neutrophil in the foreground was too opaque in the pencil drawing. To make the material appear transparent I utilized the rubber stamp set to 30% opacity to clone areas of the pannus into the explosion area.

Out of all the illustrations of my thesis work, the pair portraying the neuromuscular junction are my favorites (Figs. 4 and 6). The majority of these illustrations, including the muscle and the yellow neuron, were painted with airbrush onto Color-Aid paper and then scanned into Photoshop 2.0. The shapes of the receptors in the foreground were then masked off with the pen tool and rendered. The receptors in the middle ground and background were all the same receptor which was repeatedly duplicated, resized and rotated. The ions streaming out of the receptors were stippled in with the pencil tool.

The acetylcholine molecules started out as green and yellow spheres in a separate document. The component parts were then pasted in, each time lightly altering their juxtaposition. Straight lines connecting the two portions of the molecules completed them.

The orientation drawing for these two illustrations began with the basic cylindrical forms for the muscle cells (Fig. 3). I first attempted making a blend to show the basic form. The difficulty lay in how the linear blend worked. You must make a straight line perpendicular to the length of the cylinder. When the cylinder is at an oblique angle the perpendicular is difficult to

discern. It would have been easier to discern the perpendicular angle if I had drawn the cylinder as a horizontal, then rotated the whole image later. But this did not occur to me at the time.

The nerve fiber was outlined with the pen tool, then filled with yellow. A shadow was added with the airbrush tool. The inset was a selection pasted in from the neuromuscular junction illustrations. The dark rings, or A bands, of the muscle cell began as one circle drawn in brown. Then, the upper right portion of it was selected, duplicated and placed in a pattern, working from front to back, gradually adjusting the opacity setting to reduce contrast as distance increased.

The illustration demonstrating cross reactivity was initiated with linear blends in all of the objects, then shadow and highlights were intensified with the airbrush tool (Fig. 5). Since the antibody molecules were so large, I opted not to utilize the antibody file I had been using earlier. Instead, I drew a new one using various shades of gray line one pixel wide, each drawn adjacent to the next. Straight neutral gray objects in an otherwise full color image tend to stand out as artificial, so I set the airbrush tool to color only, selected the pink hue of the muscle, and added it to the antibody as reflected light.

What is an Antigen ?

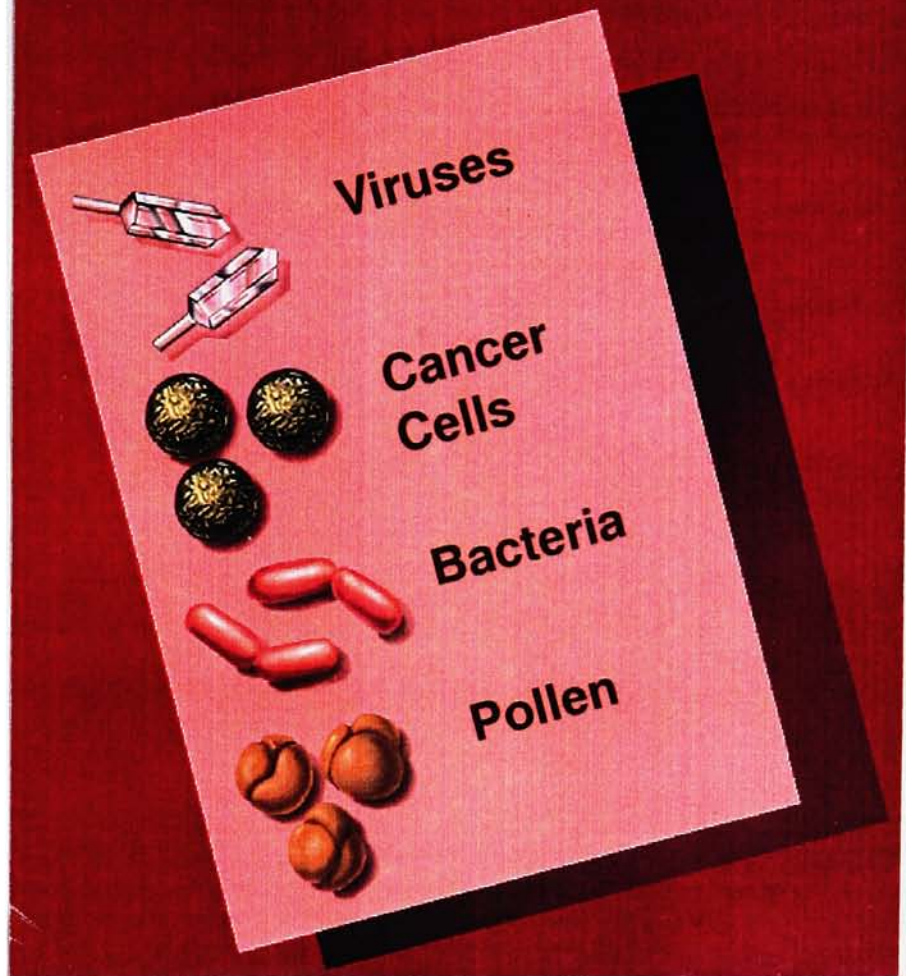


Figure 1

An antigen is any substance which is capable of evoking an immune response.



Figure 2

Depicted above are the cellular interactions of an immune response. A macrophage (yellow) engulfs some bacteria, then activates a B-cell (purple) and a T-cell (red). The helper T-cell releases chemical mediators which cause the B-cell to differentiate into the much larger plasma cell, which produces antibodies specific for that bacteria.

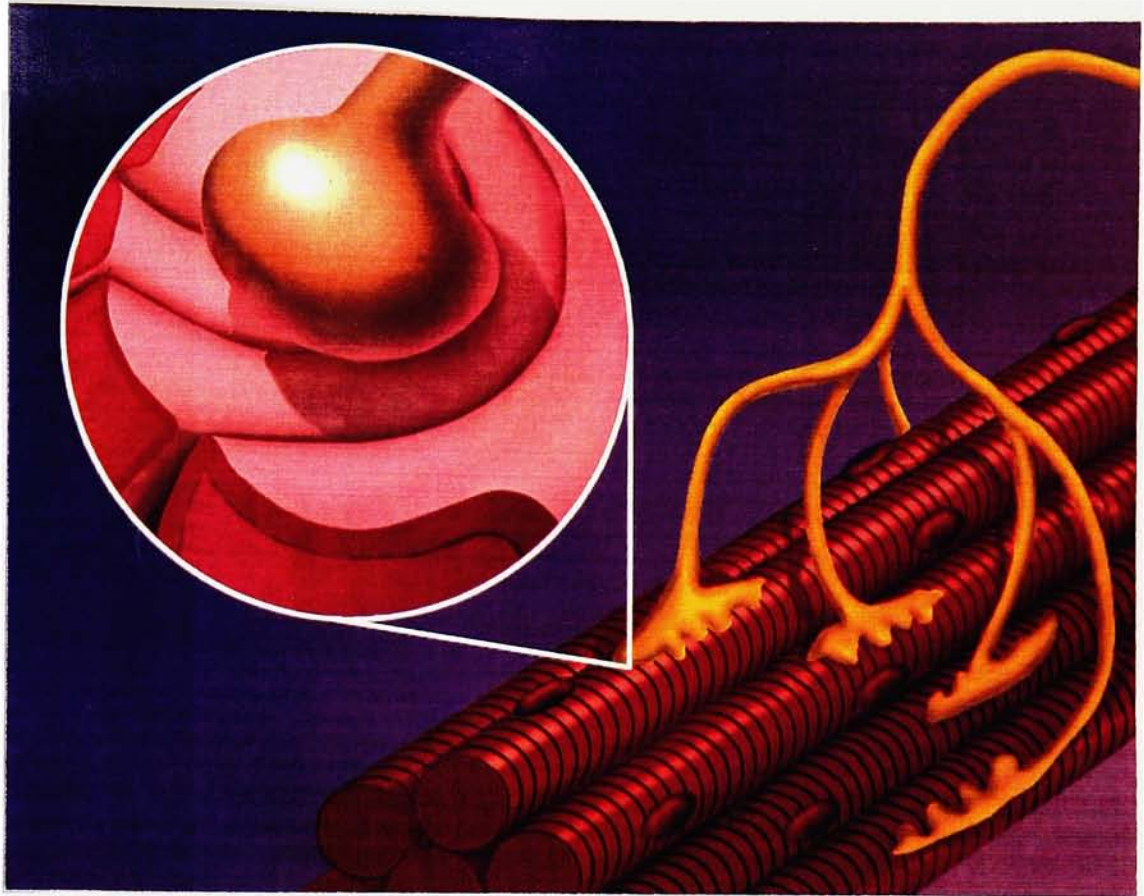


Figure 3

This is an orientation illustration designed to show the location of the neuromuscular junction. A nerve fiber branches out to innervate a number of muscle cells. The inset is a magnified view of a single neuromuscular junction.

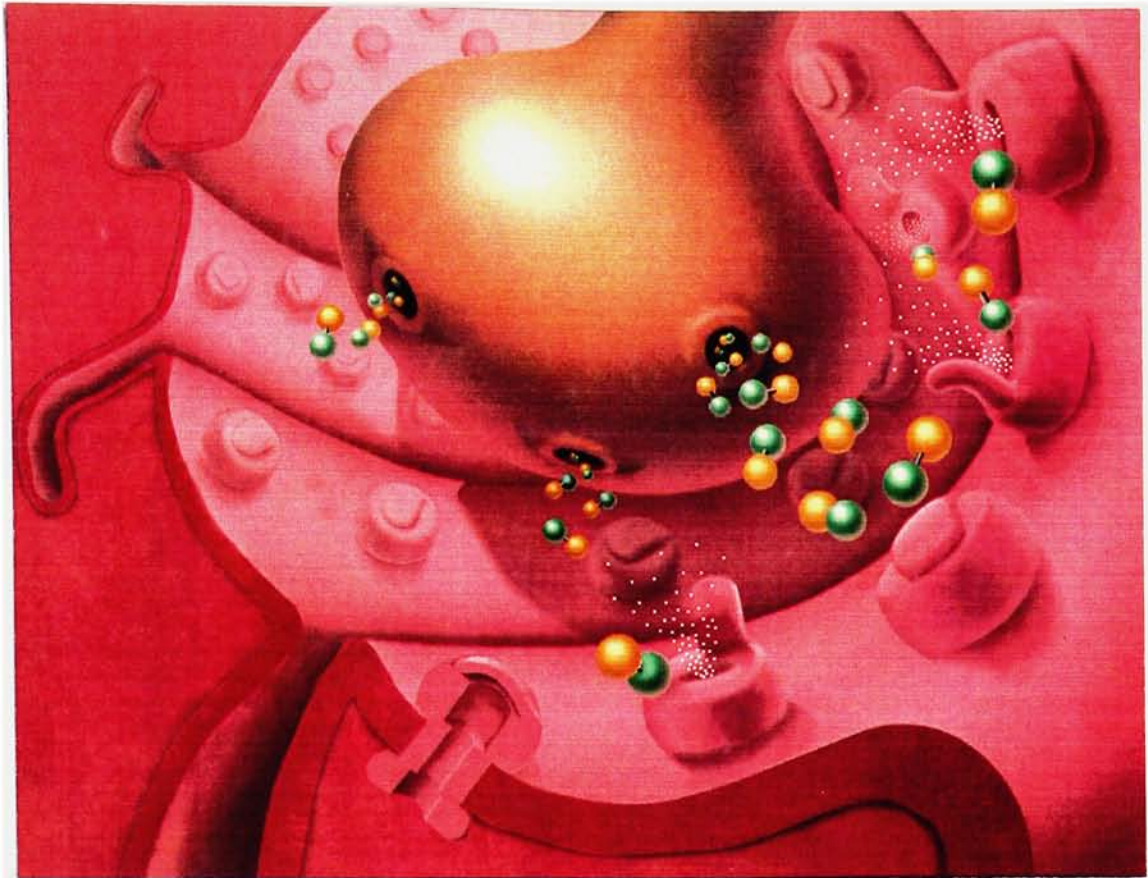


Figure 4

The terminal end of the nerve lies within an indentation in the muscle cell, called the neuromuscular synaptic cleft. The nerve may stimulate the muscle to contract by releasing acetylcholine, which moves across the synapse to bind with specialized receptors on the muscle's surface. The receptor then changes shape, allowing ions to stream in and out.

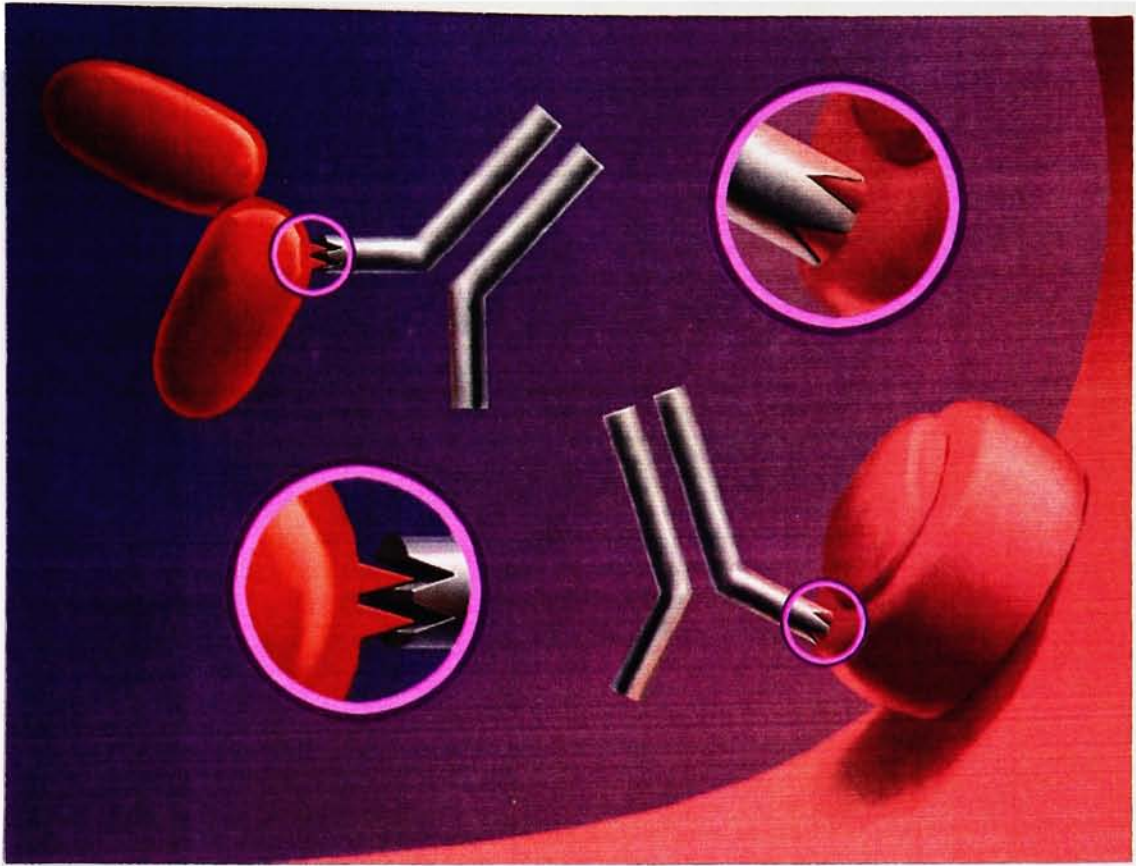


Figure 5

Myasthenia gravis is believed to be caused by a process called cross reactivity. Antibodies are produced against an antigen that, by coincidence, is very similar in chemical structure to the acetylcholine receptors of muscle cells. These antibodies bind to the receptors, thereby incapacitating them.



Figure 6
Antibodies bind to acetylcholine receptors during myasthenia gravis. Not only does this block the receptors from binding to acetylcholine, but also it promotes their endocytosis by the muscle cell.

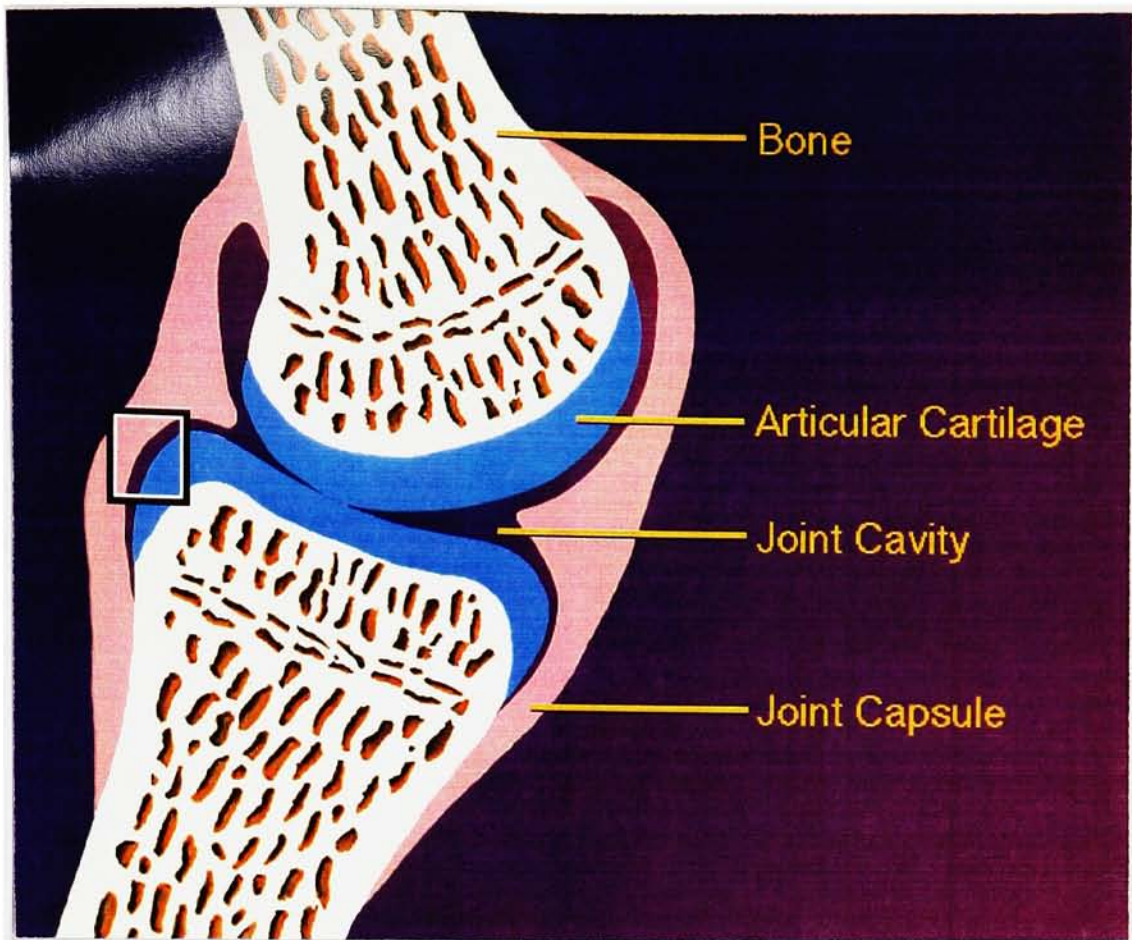


Figure 7

The synovial joints are the most typically involved region of the body in rheumatoid arthritis. This cut away view shows the major structures of the synovial joint. The region within the small black rectangle is magnified in figures 9 and 12.

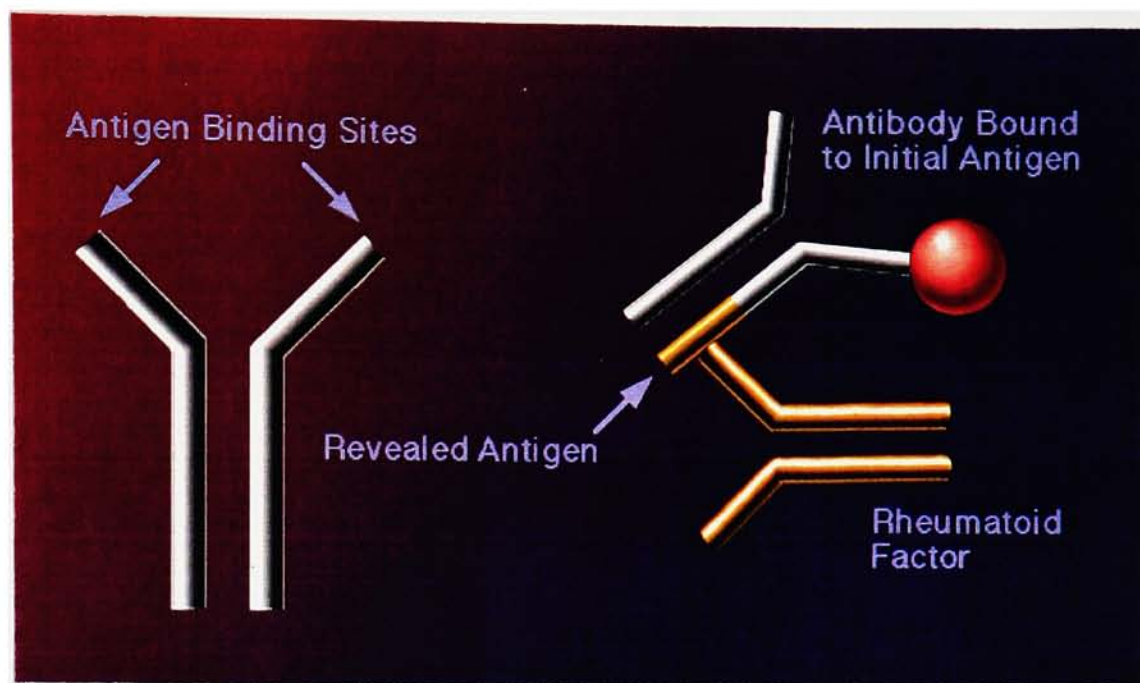


Figure 8

Antibodies are protein molecules which bind with an antigen as part of the immune response. Normally, the binding sites of the antibody attach to an antigen as a step towards the antigen's destruction. In rheumatoid arthritis, an antibody binds to an unknown antigen that is capable of transforming the structure of the antibody. A region of the antibody is revealed which the immune system interprets as a foreign antigen. The immune system then synthesizes rheumatoid factors, which are antibodies that bind to the altered antibodies. When antibodies and antigens are bound together, they are called immune complexes.

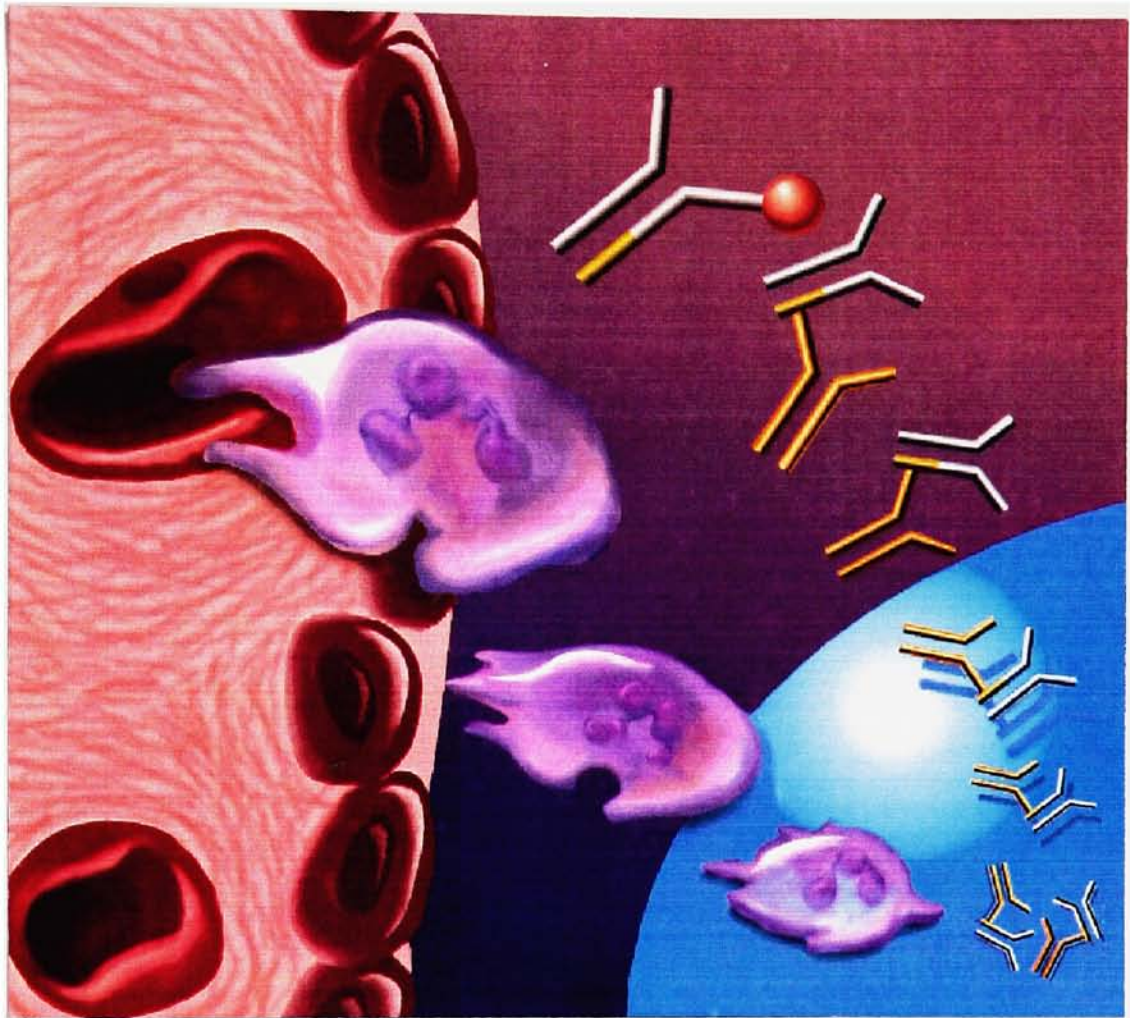


Figure 9

The immune complexes of rheumatoid arthritis tend to settle within articular cartilage. Phagocytic cells from the blood, called neutrophils, move towards the articular cartilage after chemically sensing these aggregated complexes.



Figure 10

Neutrophils normally attempt to engulf immune complexes before they release their digestive enzymes. However, if the immune complexes are sequestered within the cartilage matrix, neutrophils release enzymes directly onto the cartilage. These enzymes damage the collagen fibrils composing the cartilage.

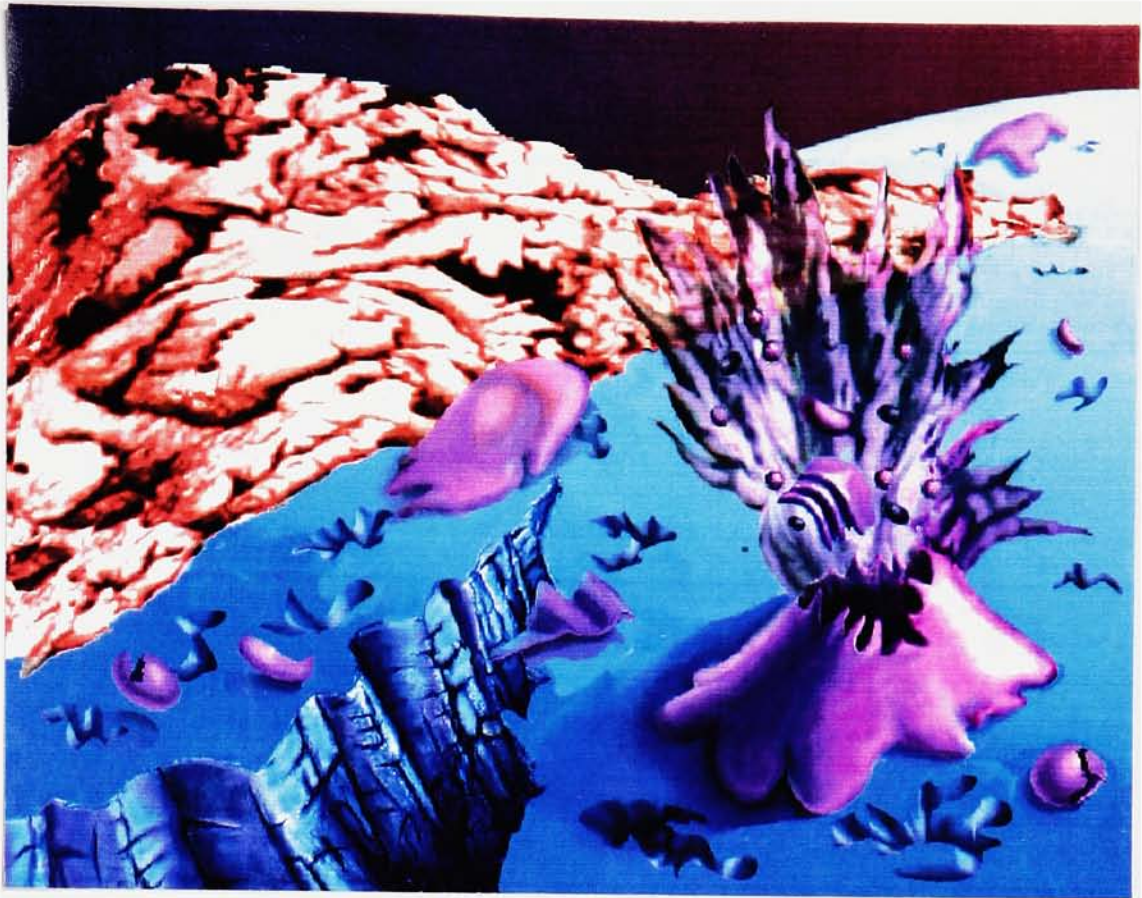


Figure 11

This is a microscopic panoramic view of the articular cartilage undergoing destruction from rheumatoid arthritis. Neutrophils move across the corroded surface in search of sequestered immune complexes. The neutrophils continue to ingest material until they burst open. The background at left shows a formation of pannus, an ingrowth of the synovial membrane which can damage cartilage and fuse joints.

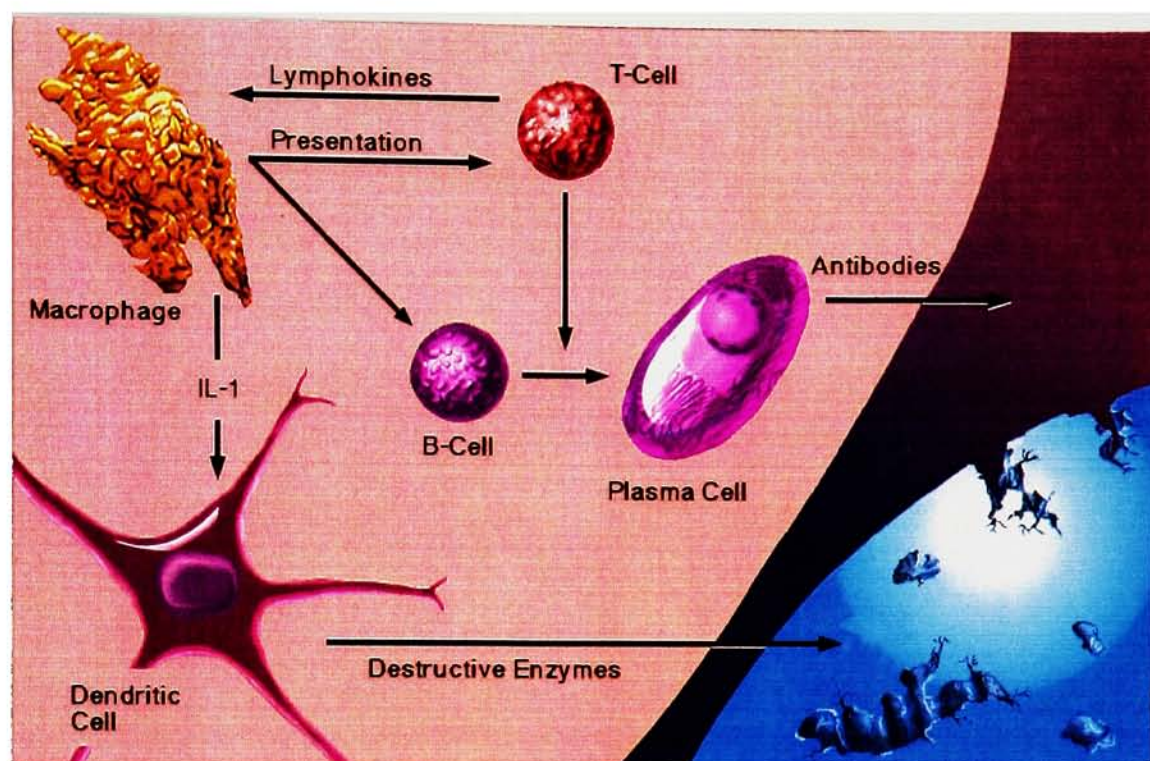


Figure 12

This illustration shows some of the chemical interactions between cells within the joint capsule. Macrophages activate helper T-cells through antigen presentation. The helper T-cells attract more macrophages by releasing lymphokines. Lymphokines induce macrophages to release IL-1, which in turn causes the dendritic cells to release enzymes that are destructive to cartilage. The T-cells and macrophages work in concert to promote the B-cells to differentiate into plasma cells. The plasma cells produce antibodies which add to the formation of new immune complexes, perpetuating the inflammatory response.

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